Further Survey Results on Xenoestrogen Exposure of Benthic Fish in Puget Sound

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Abstract

Vitellogenin (Vtg), a yolk protein produced in the liver of oviparous animals in response to estrogens, normally occurs only in sexually mature females with developing eggs. However, males can synthesize Vtg when exposed to exogenous estradiol or to substances that mimic estrogens. Thus, the abnormal production of Vtg in male animals can be used as a biomarker for exposure to estrogenic substances. In 1997 through 1999, as part of the Puget Sound Ambient Monitoring Program (PSAMP), plasma from adult male English sole from numerous urban embayments in Puget Sound were analyzed for the presence of Vtg utilizing a quantitative enzyme-linked immunosorbent assay (ELISA) for this species. Results from the project revealed the presence of significant levels of Vtg in sole from several urban sites. Although no significant correlation was found with contaminant (e.g., PAHs, PCBs) levels in bottom sediments, the highest Vtg concentrations and greatest percentage of fish that exhibited Vtg were from sites near combined sewage overflows (CSOs). Between 2000 and 2002 the study was expanded to include several new sites and conduct follow-up surveys at several 'sites of concern' to better assess the spatial and temporal trend of Vtg presence in male sole. These new results will enable us to determine the extent to which xenoestrogen exposure is occurring in Puget Sound marine fish.

Introduction

Endocrine disrupting chemicals (EDCs) is a term applied to any natural or man-made substance capable of acting as a hormone mimic or blocking hormone action. These substances can interfere with the production, release, transport, metabolism, binding, action or elimination of an organism's natural hormone (Kavlock et al. 1996). This interference can alter the natural hormonal balance in the animal by disrupting normal endocrine system responses and functions, causing potential adverse effects on the development, growth, behavior and reproduction at the individual and population level (Ankley et al. 1998).

Xenoestrogens are one group of EDCs that have received an enormous amount of attention because they are known to interfere with estrogen responses crucial for normal development and reproduction. The structures of these compounds are very similar to naturally occurring estrogens in the body and include compounds such as synthetic estrogens (estrone, ethinyl estradiol), surfactants (alkylphenol ethoxylates), plasticizers (bisphenol-A) and phytoestrogens (β-sitosterol). Some of these compounds are persistent and can bioaccumulate; other EDCs may have additive effects. They are often associated with sewage outfalls and industrial sites. Studies suggest that these compounds are widespread in US freshwater systems (Kolpin et al. 2002); however, considerably less is known about their distribution in marine waters such as Puget Sound.

Fish exposed to xenoestrogens in the wild and in laboratory studies have shown a wide variety of effects including inhibited testis growth, reduced sperm production and quality, intersex fish, reduced egg production, altered reproductive timing and behavioral changes. Perhaps one of the most studied biological responses to xenoestrogens is abnormal vitellogenin (Vtg) production in male fish. Vtg is a sex-specific protein synthesized in the liver of oviparous animals in response to circulating estrogens. Although vitellogenesis normally occurs only in sexually mature females, it can be induced in males by exposure to estrogens or to substances that mimic estrogen, making it a useful biomarker for exposure to xenoestrogens. Although uncommon, several cases of Vtg production in wild male fish (both marine and freshwater) have been documented in rainbow trout, roach and flounder (Purdom et al. 1994; Harries et al. 1996, Allen et al. 1999, Hashimoto et al. 2000).

Vtg is relatively easy to measure in blood plasma using immunoassay techniques such as enzyme-linked immunosorbent assay (ELISA). We developed (Lomax et al. 1998) an ELISA to measure plasmatic vitellogenin (Vtg) in English sole (*Pleuronectes vetulus*), a benthic marine fish that are particularly sensitive to chemical contaminants (Collier et al. 1992; Myers et al. 1994, Stein et al. 1992; Johnson et al. 1998). These studies demonstrated that English sole inhabiting contaminated sites are prone to various kinds of reproductive dysfunction, which may be related to exposure to xenoestrogens or anti-estrogenic compounds (Johnson et al. 1998). During 1997-1998 blood plasma was collected from male English sole as part of the Puget Sound Ambient Monitoring Program (PSAMP). The results of this study revealed that xenoestrogens were indeed present and available for uptake by bottom fish in Puget Sound. Subsequent sampling

in recent years (1999-02) at the established sites and some new sites showed that xenoestrogens are present throughout much of Puget Sound and appear to be most prevalent at sites near sewage and combined storm water/sewage outfalls (CSOs).

Methods

All the fish used in this study were collected by bottom trawl during the spring of each year (April/May) from numerous urban and non-urban embayments throughout Puget Sound. Blood was withdrawn from the caudal vein of adult male (total length 200-350mm) English sole (3-15 years of age as determined by otolith) using heparinized syringes. After centrifugation, plasma was collected and treated with the protease inhibitor phenyl methyl sulfonyl sulfate (PMSF, 1mM in plasma) and then frozen at –80°C until analysis. Plasma samples were analyzed for the presence of Vtg utilizing a quantitative ELISA for this species. This assay uses purified English sole Vtg as a standard and a polyclonal antibody developed in rabbits against the English sole Vtg. The ELISA is a competitive antibody capture assay and is described in detail elsewhere (Lomax et al. 1998). The number of samples analyzed for each site/year ranged from a low of 8 at Port Susan (2001) to a high of 33 at the Harbor Island (2002) site. Most of the sites had samples sizes of 17-25 fish.

Results

The sites sampled during this study include 5 each within Elliott Bay, Sinclair Inlet and Commencement Bay plus Nisqually Reach, Eagle Harbor, Port Gardner, Hood Canal and Port Susan. Table 1 shows a list of the 20 sites and when they were sampled. For the sake of simplicity, the sampling effort can be broken into 2 time periods: Survey 1 (1997-1998) and Survey 2 (1999-2002). Most of the sites were sampled at least once during Survey 1. Survey 2 included Eagle Harbor, a focused effort in Commencement Bay and a follow-up of those sites that were identified during Survey 1 as ‘sites of concern’.

Table 1. The Puget Sound Ambient Monitoring Project (PSAMP) sites sampled for this study and the years in which male English sole blood plasma samples were collected and analyzed for vitellogenin.

Site name	Year Sampled
Elliott Bay Focus	
• Seattle Waterfront	1997, 2002
• Harbor Island	1997, 2001, 2002
• Duwamish Waterway	1997
• Myrtle Edwards Park	1997, 2001, 2002
• Alki Point	1997
Sinclair Inlet Focus	
• Bremerton Waterfront	1997, 1998
• Outer Sinclair Inlet	1998
• Port Orchard South	1998
• Port Orchard North	1998
• Blake Island	1998
Commencement Bay Focus	
• Thea Foss Waterway	1997, 1999, 2001
• NE Commencement Bay	1999
• Ruston	1999
• Old Tacoma	1999
• Browns Point	1999
Eagle Harbor	2001, 2002
Nisqually Reach	1997
Port Susan	1997, 2001, 2002
Port Gardner	1997
Hood Canal	1997, 2002

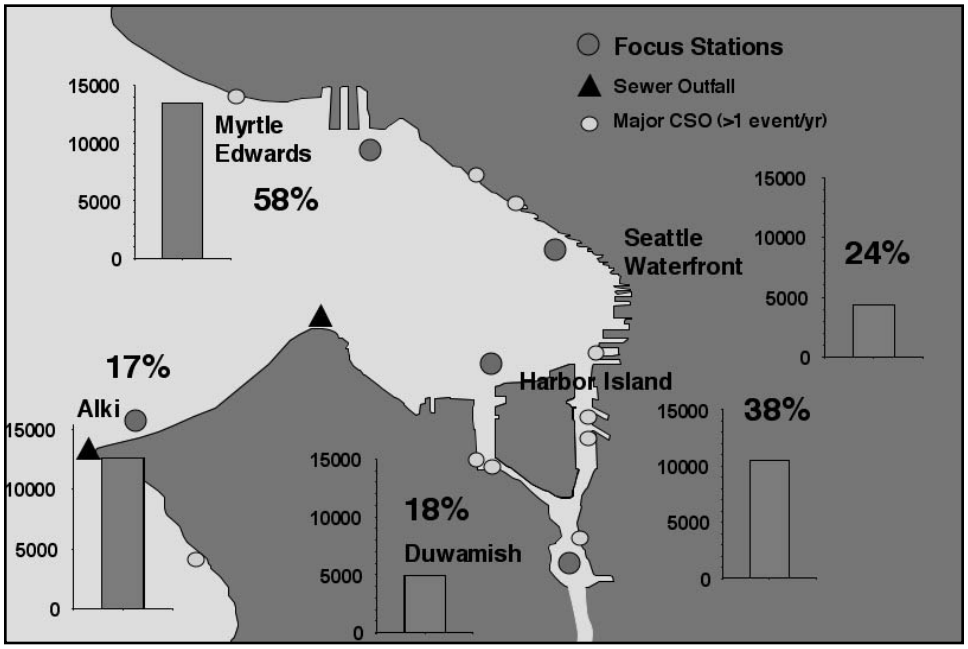

Figure. 1 Percentage of fish affected and average concentration of vitellogenin (ng/ml plasma) in male English sole from Elliott Bay, Washington. The locations of sewer outfalls and CSO's are also shown.

Survey 1

Fish sampled from most urban areas had quantifiable levels of Vtg and concentrations ranged between 2-70ug Vtg per ml of plasma. Elliott Bay was a focus site in 1997 and all five stations within Elliott Bay had fish with Vitellogenin (Fig. 1). The Myrtle Edwards site had both the highest percentage of animals affected (58%) and highest average concentration (13 µg/ml). This station is in close proximity to 3 major CSOs, which are potential sources of environmental estrogens. The Harbor Island site also had a high percentage of fish affected and high Vtg levels. The 3 remaining sites in Elliott Bay all showed similar proportions of fish with Vtg (~20%), however the Alki Point fish had the highest Vtg levels.

Outside of the Elliott Bay sites, the only other stations where more than 20% of fish had Vtg were the Thea Foss Waterway in Commencement Bay and the Bremerton Waterfront site. Both of these sites are located in highly industrialized areas with CSOs and sewer treatment plant (STP) outfalls nearby. Port Gardner was another urban station that had a few fish with Vtg. The presence of Vtg was also discovered in some fish from two non-urban stations, Port Susan and Blake Island. Vtg was not detected at the other non-urban stations in Hood Canal and Nisqually Reach.

Survey 2

Based on our findings from the first 2 years, it was determined that the Myrtle Edwards and Harbor Island sites in Elliott Bay needed to be visited annually if possible. The results from the Thea Foss Waterway suggested that Commencement Bay was an 'area of concern' and it was a focus site in 1999. Eagle Harbor station was added and several of the other non-urban sites were revisited annually beginning in 2001. The results from the Survey 2 continue to show that xenoestrogens are present in Elliott Bay. The Myrtle Edwards, Harbor Island and Seattle Waterfront sites all continue to have greater than 30% of male English sole with measurable Vtg and an average concentration of 10 µg/ml plasma. A more detailed study in Commencement Bay showed the Ruston station to have the highest percentage of animals affected (31%) and highest average concentration (14 µg/ml) (Fig. 2). The Thea Foss Waterway continued to produce vitellogenic males, though the percentage of fish was considerably less than Survey 1. The only other site in Commencement with affected fish was the Outer Bay site. The Eagle Harbor site did not have a single fish with Vtg among the 45 samples analyzed in the 2001-2002 period. For the non-urban stations where sampling continued, Port Susan and Hood Canal had a combined total of only 1 fish with Vtg (n=60).

Conclusions

Results of the initial pilot study (Survey 1) clearly demonstrated that xenoestrogens are present and available to Puget Sound bottom fish. The presence of vitellogenic male English sole at numerous sites around Puget Sound indicates that

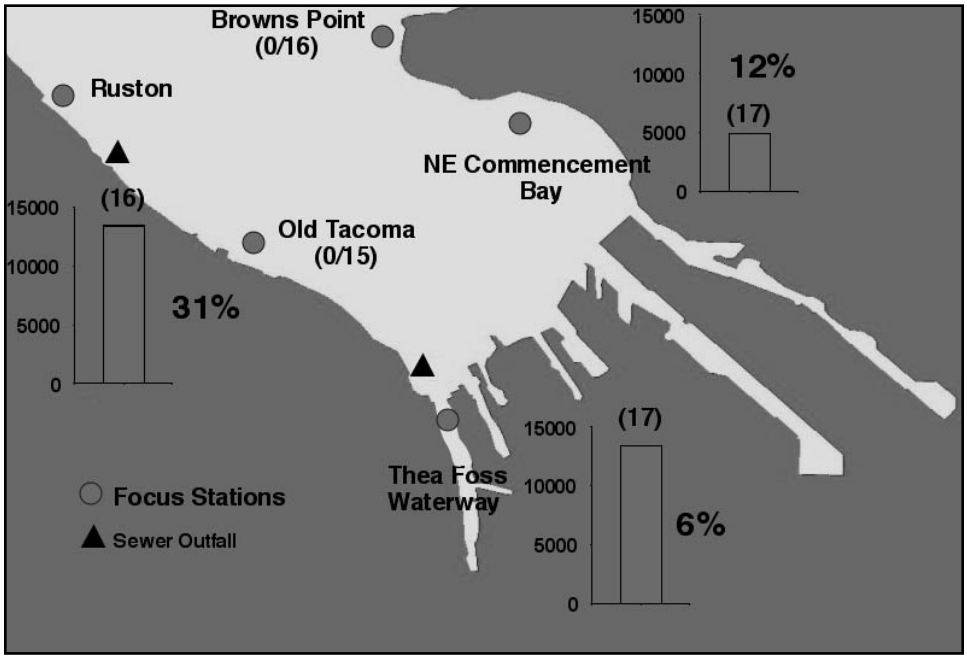

Figure 2. Percentage of fish affected and average concentration of vitellogenin (ng/ml plasma) in male English sole from Commencement Bay, Washington. The locations of sewer outfalls are also shown.

these fish were exposed to estrogen-like compounds. We have not determined if other species are affected. With 6 years of data covering 20 stations, we are able to draw some conclusions about how widespread the problem is in Puget Sound. Xenoestrogens appear to be most prevalent near urban and industrial centers, usually near CSO and/or sewage treatment plant (STP) discharges. The Myrtle Edwards station had the highest overall percentage of fish exhibiting Vtg production and is near several major combined sewer overflows (CSO). These same characteristics apply to other sites with high percentages of fish and/or high Vtg levels such as Harbor Island, Seattle Waterfront and several Commencement Bay stations. The exposure also appears to be consistent over time, as the percentage of fish affected and average Vtg levels at these urban sites has not changed dramatically during the 6 years.

In contrast, xenoestrogens appear to be less of a concern at non-urban sites, but were occasionally detected. For example, the discovery of vitellogenic fish from Port Susan was unexpected in Survey 1. This finding may be explained by the presence of two small sewage treatment plant discharges in the area, that the Port Susan area was believed to have many failing septic systems and also receives agricultural sources of pollutants. Port Susan is also a relatively confined embayment with minimal currents that do not easily flush pollutants from the area. Interestingly, subsequent sampling from Port Susan in 2001-2002 revealed zero fish with Vtg, suggesting that perhaps the source of xenoestrogens in 1997 might have been removed or cleaned-up. Other non-urban sites such as Hood Canal and Nisqually Reach typically had very few, if any fish with measurable Vtg levels.

The question of whether exposures are high enough to affect fish health remains largely unanswered. The levels of Vtg seen in these fish are comparable to levels found in other species from studies where biological effects such as intersex and altered testis shape were observed (Fig. 3) (Folmar et al. 1996; Jobling et al. 1998, Allen et al. 1999, Hashimoto et al. 2000). Studies on English sole from Elliott Bay, WA suggest that female fish were exhibiting delayed development (personal communication, S.M. O'Neill). A large proportion of the females from Elliott Bay sites had developed hydrated eggs and were ready to spawn in April and May (Fig. 4), well past the normal spawning cycle observed (Jan. - Mar.) for English sole at other sites in Puget Sound. This delay in spawning time could reduce the likelihood of egg fertilization and larval survival. Accordingly, a more intensive sampling effort was conducted this year in Elliott Bay to better characterize this reproductive anomaly and its possible cause. Future research efforts will study the relationships between xenoestrogen exposure and other potential biological effects in male sole. In addition, we are interested in studying other target species and recently initiated a project that monitors juvenile outmigrant chinook and coho salmon at many of these same sites.

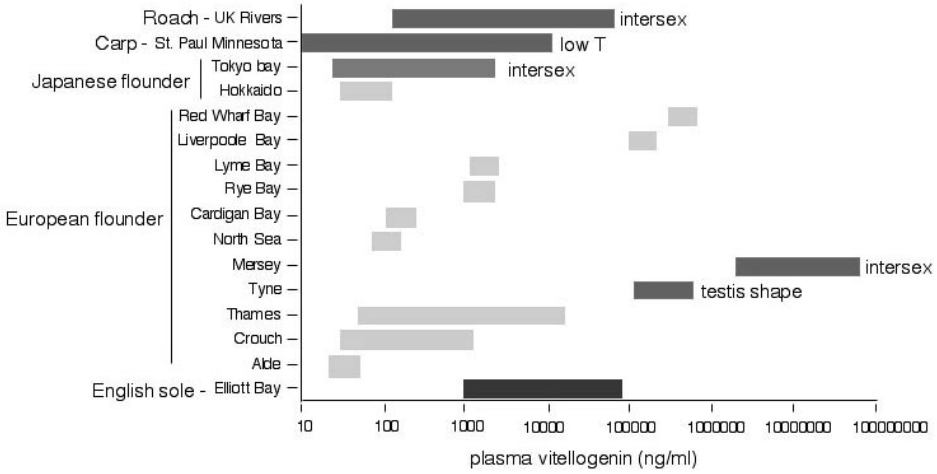

Figure 3. Vitellogenin levels in Elliott Bay English sole compared to levels measured in other fish species where biological effects were observed.

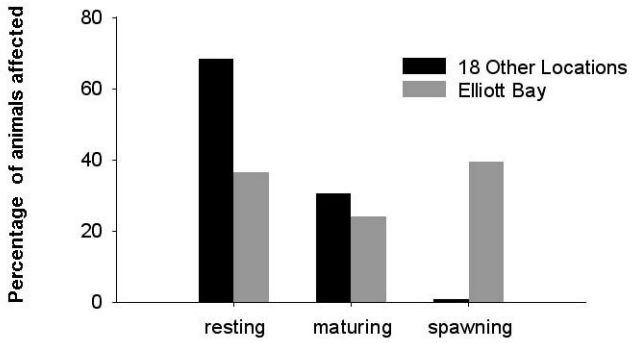

Figure 4. Percentage of fish at different stages of maturity. A comparison of female English sole collected in April/May from Elliott Bay vs. the rest of Puget Sound.

While our findings from this study indicate that English sole are exposed to xenoestrogens in Puget Sound, we have no information on what contaminants are responsible. The results suggest that exposure to these chemicals may be associated with sewage or other industrial waste. Sewage effluent markers such as fecal coliform, coprostanol and caffeine were not measured as part of the present study, nor were other chemicals with known estrogenic activity such as alkylphenols measured in sediments or in fish. However, studies are currently planned to identify and measure the chemicals in either water or sediments that could be active agents. The identification of the chemicals responsible for vitellogenin induction in male English sole would be a major step towards discovering the source of the contaminants, and ultimately could lead to their cleanup and removal from these urban waterways.

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